NEW BAKER'S YEASTS AND STRAINS FOR THEIR PREPARATION

The present invention relates to new baker's yeasts or bread-making yeasts. It also relates to strains for the preparation of said baker's yeasts.

In the USA, the trade of frozen doughs, notably of sweet doughs such as frozen doughs intended for bakery products called "Rolls" ~~or~~, for Danish sweet pastries or for similar sweet fermented and baked products is quickly expanding ; however, freezing involves an important stress for the yeast.

In the USA, small breads or fermented pastries are very often aromatized (flavored) with cinnamon ; this spice contains cinnamic acid and cinnamaldehyde ; these chemical compounds can be metabolized by yeasts which leads to the appearance of bad taste and of bad flavors, also called "off-flavors".

~~Are already known baker's~~Baker's yeasts are known that ~~which~~ are resistant to the stress caused by freezing or deep-freezing and which can be used in frozen doughs without the necessity ~~to increase~~of increasing ~~in~~ to an important extent the amount of yeast[[,]] ~~increase which~~ as is necessary when using conventional baker's yeasts ~~which~~ that are not resistant to ~~said~~ the stress caused by freezing or deep-freezing. Baker's yeasts resistant to freezing have been developed in Europe and in Japan.

Also known are baker's yeasts which can be used without formation of off-flavors, in the manufacture of bakery products comprising cinnamon. Such baker's yeasts, which do not give rise to the appearance of off-flavors in the presence of cinnamon, are commonly marketed in the USA.

The new baker's yeasts ~~which the applicants had the merit of having developed~~of the present invention have the properties of the two types of baker's yeasts here-above discussed.

These new baker's yeasts are characterized by the fact that :

- they have good general ~~performances~~ performance in not delayed bread-making processes, i.e., in bread-making processes ~~which~~ that do not comprise a freezing or deep freezing step,

- they are resistant to the stress caused by freezing when they are used in sweet doughs and,
- they do not give rise to the appearance of bad taste or of off-flavors in the presence of cinnamon.

Indeed this property of not giving rise to the appearance of bad taste or of off-flavors is essential for a baker's yeast and must be verified in any breadmaking process wherein the said baker's yeast is intended to be used.

In the tests ~~A1~~A_1, ~~A5~~ A_5 and ~~A6~~ A_6 carried out with the fermentometer of Burrows and Harrison, ~~and which are~~ described in column 5 of US ~~patent~~ Patent No. 5,741,695 of April 21, 1998, the entire disclosure of which is incorporated by reference, ~~the said~~ good general ~~performances~~ performance in not delayed bread-making processes obtained with the ~~said~~ new baker's yeasts of the present invention ~~lead~~ led to the following gas releases:

- in test A_1, at least 150 ml in 2 hours,
- in test A_5, at least 90 ml in 2 hours,
- in test A_6, at least 80 ml in 2 hours.

In other words, ~~these~~ the new baker's yeasts of the present invention are characterized by the fact that in not delayed bread-making processes, they give rise in the fermentometer tests A_1, A_5 and A_6 carried out with the fermentometer of Burrows and Harrison to gas releases which are at least equivalent ~~with~~ to those obtained with a control yeast produced according to a conventional process starting from the strain deposited at the "Collection Nationale de Cultures de Microorganismes" (CNCM), Institut Pasteur under the number CNCM I-2412, on March 22, 2000, ~~said strain being~~ which is representative of the strains commonly used in the USA for the manufacture of baker's yeasts.

A conventional process of baker's yeast manufacture is a process described in ~~the chapter~~ Chapter 6: "Baker's Yeast Production" of the handbook "Yeast Technology", Second Edition, Reed and Nagodawithana, An Avi Book published by Van Nostrand Reinhold, 1991.

The resistance toward ~~the~~ stress caused by freezing of the new baker's yeasts according to the invention is characterized:

- on the one hand by the fact that, when used in doughs corresponding to formulations of sweet Danish pastries, i.e., to doughs comprising about 18% sugar (sucrose) by weight with respect to the flour used and comprising fats, the total gas releases recorded after freezing and thawing of the said doughs after at least 100 days are higher by at least 20 %, preferably by at least 30 % and still more preferably by at least 40 % than the total gas releases recorded under the same conditions when using a control yeast obtained conventionally starting from a control strain such as the strain CNCM I-2412, and
- on the other hand by the fact in the above-defined use, the proof time of the said sweet Danish pastry dough, frozen and thawed after at least 100 days, is lower by at least 10 %, preferably by at least 15 % and still more preferably by at least 20 % than that measured under the same conditions when using the above-defined conventional control yeast.

~~Indeed in~~In these comparisons the control yeast must ~~be under~~have the same form ~~than~~ as the new baker's yeast of the present invention that is tested.

Preferably, the ~~said~~ total gas releases on the dough pieces are measured using the zymotachygraphe CHOPIN® ~~during~~ for 2 hours and 30 minutes at 27°C and the proof times are measured at 35°C.

~~It is recalled that the proof~~Proof time as used herein is ~~according to the handbook of basic technical baking terminology by E.J. Pyler used as reference~~ the length of time for which a moulded dough piece is held in the final proofer prior to baking so it can attain the desired degree of aeration or volume increase. See, The Handbook of Basic Technical Baking Terminology by E.J. Pyler.

It is recalled that the zymotachygraphe CHOPIN® or CHOPIN® zymotachygraphe is a conventional apparatus known to those skilled in the art for measuring the gaseous release of a dough ball or piece. This apparatus is notably described in a detailed manner in chapter VII B "Appreciation du pouvoir fermentaire" (appreciation of the fermenting power), §6.5 "Le Zymotachygraphe" (CHOPIN, 1973),

pages 461 to 463 in the manual "Guide pratique d'analyses dans les industries des céréales", B. Godon and W. Loisel, Technique et Documentation (Lavoisier) 1984, ISBN 2-85206-081-7 Collection 2-85206-230-5. The fermentometer of Burrows and Harrison is the object of §6.1 of this Chapter VIIB, pages 454 to 460.

For the preparation of the new baker's yeasts according to the invention, it is possible to use two strains which were deposited on the 24th of March 2000 according to the Budapest Convention with the "Collection Nationale de Cultures de Microorganismes" (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 PARIS CEDEX 15, FRANCE, under the numbers:

I-2421 (strain L17)

I-2422 (strain L35).

Strain ~~now called~~ L17, ~~in~~ for the reason of its possible industrial use ~~and~~ was deposited under the number CNCM I-2421 and had been cited in a different context in US ~~patent~~ Patent No. 5,741,695, but the ~~said~~ strain had been considered at that time as presenting no interest as a baker's yeast strain due to its ~~too~~ high invertase content. It ~~has~~ was not ~~been~~ deposited before the 24th of March 2000 with a Public Culture Collection Center (deposit according to the Budapest Convention). It has always been kept in the private and confidential collection of the Lesaffre Group in its research laboratories located at 59700 Marcq-en-Baroeul in France without being commercially or publicly used and without being accessible to third parties. Consequently, this strain is a novel baker's yeast strain.

Strain L35, deposited under the number CNCM I-2422 was obtained by mutation starting from strain L17; that strain has never been cited in a prior art document, and is also a novel baker's yeast strain.

These two strains were selected from a great number of strains among ~~especially~~ the strains of the private collection of the Lesaffre Group using three series of systematical selection tests. The confidential and private collection of the Lesaffre Group contains numerous strains which were constructed, notably according to the reproducible construction processes of new strains disclosed by the US ~~patents~~ Patent

Nos. 4,396,632 and 5,741,695. The entire ~~disclosure~~ disclosures of these two US patents ~~is~~ are incorporated herein by reference.

In these different series of tests, the control strain is the one which was deposited with the "Collection Nationale de Cultures de Microorganismes", under the number I-2412. That strain is representative of the strains which are commonly used in the USA for the manufacture especially of baker's yeasts intended for the breadmaking of products of the sweet roll type and of Danish pastries.

Selection Tests

~~That~~ The control strain and the various tested strains ~~had been~~were cultivated on cane molasses in pilot installations. The scheme of the cultivation on cane molasses, used here, is the one disclosed in example 3 of US patent 5,741,695 from line 40, column 12, to line 26, column 13. ~~It has permitted to obtain fresh~~Fresh baker's yeasts having a dry matter content of about 32% were obtained. The nitrogen content with respect to the dry matter of these fresh yeasts is adjusted between about 8.2% and about 8.5%.

Test S1

~~In a first time, the~~The first selection test, S1, consists ~~in~~ of searching the various fresh baker's yeasts obtained with the various tested strains that give rise in tests A_1, A_5 and A_6 carried out with a fermentometer of Burrows and Harrison as disclosed in column 5 of ~~the~~ US ~~patent~~ Patent No. 5,741,695 which is incorporated herein by reference, to results which are at least 150 ml in test A_1 in two hours, at least 90 ml in test A_5, in two hours and at least 80 ml in test A_6, in two hours.

~~It is being recalled that all these~~These results, in absolute value, concerning gas releases or proof times, must always be compared with respect to at least a control. As a matter of fact, the principles indicated in example 6 of US ~~patent~~ Patent 5,741,695, columns 19 and 20, and especially column 20, lines 50 to 57 have a general value.

Test S2

~~Then, the~~The second systematical selection test, S2, consists ~~in~~ of the examination ~~from the point of view~~ of their behavior with respect to cinnamon, of fresh

yeasts issued from the first selection test, i.e. fresh yeasts, which, in the tests A_1, A_5 and A_6, give at least the hereabove defined results.

In ~~that connection~~the second selection test, S2, , quantities of 150 mg of dry matter of each of the ~~said~~ fresh baker's yeasts ~~are used in order to ferment during~~fermented for 4 hours at 30°C[[,]] under weak agitation in two 125 ml flasks that are not hermetically closed and that contain respectively 20 ml of each of ~~two~~ a first and second sweet nutrient ~~solutions under weak agitation in 125 ml flasks not hermetically closed~~solution. The two nutrient ~~solution~~solutions, buffered to pH 5.5, ~~contains~~ contain ~~in a total volume of~~per 1000 ml: 4.7 g $(NH_4)_2HP0_4$, 2 g Mg SO_47H_2O, 0.8 g KCL, 10 ml vitamins solution and 150 ml citrate buffer. The 10 ml solution of vitamins contains 4 mg thiamin (B1 vitamin), 4 mg pyridoxyn (B6 vitamin) and 40 mg nicotinic acid. The 150 ml citrate buffer contains 14.14 g of trisodium citrate and citric acid necessary to adjust the pH to 5.5. The first sweet nutrient solution or control solution consists of the ~~said~~ nutrient solution to which is added 6% of sorbitol, 0.25% of yeast extract and 6% of glucose. The second sweet nutrient solution consists of the ~~said~~ nutrient solution to which is added 6% of sorbitol, 0.25% of yeast extract, 6% of glucose and 0.04% of cinnamic acid. The percentages are expressed in weight with respect to the total or final volume of sweet nutrient solutions.

——The second test consists in the comparison of the odor of the solution fermented without cinnamic acid with that of the solution fermented in the presence of cinnamic acid. This test of comparison of odors, based on the detection of off flavors due to the decomposition of cinnamic acid is made on the basis of notes given by a jury. The notes given by the jury can be confirmed by ~~analyses~~ analysis of the decomposition rate of cinnamic acid, i.e., by the determination of the cinnamic acid still present at the end of the test and/or by the determination of styrene present in the solution fermented in the presence of cinnamic acid.

The presence of Cinnamic acid and styrene can be ~~dosed~~ measured by chromatographic methods known to those skilled in the art in the fermented solutions centrifuged at 4°C in order to remove yeast cells. The presence of cinnamic acid can be ~~dosed~~ measured by reverse phase HPLC (High Performance Liquid Chromatography)

on C18 column, the elution being realized by a gradient of acetonitrile between 10% and 40% in water, in presence of 0.1% of trifluoroacetic acid (percentages volume/volume), the detection being realized by an U.V. detector at 260 nm. The presence of styrene can be ~~dosed~~ measured by gas chromatography coupled with mass spectrometry. The presence of styrene was ~~dosed~~ measured in a VARIAN® GC 38000 gas chromatograph equipped with column CHROMPACK® CP-Wax 52 CB 30m*0.25mm, df:0.5μm. The sampling ~~methods~~ method used was based upon static headspace, wherein 3 g of supernatant ~~being~~ was placed into 10 ml glass vials which ~~are~~ were heated at 35°C with agitation for the timed equilibrium step (15 min), and the headspace volume of 100 μl ~~being~~ was injected into the GC column. The oven temperature of the chromatograph was programmed as follows: 2 min isotherm at 55°C, heated at 5°C/min up to 230°C. Helium was used as carrier gas with a flow rate of 1 ml/min. The styrene was detected by mass spectrometry SATURN 2000 VARIAN®.

This second test shows that most of the baker's yeasts used in Europe, and especially the baker's yeasts which in Europe are considered as efficient on frozen doughs, give rise to the appearance of off-flavors in the presence of cinnamic acid.

At the end of this second test, only ~~are selected~~ baker's yeasts ~~which~~ that do not give rise to the formation of off-flavors are selected. Notably, baker's yeasts obtained from the strain L17 (CNCM I-2421) and from the strain L35 (CNCM I-2422) pass successfully this second test.

The control baker's yeast obtained starting from the strain CNCM I-2412 does not give rise to the formation of off-flavors in that test.

The strains CNCM I-2412, CNCM I-2421 and CNCM I-2422 ~~being~~ are three examples of strains which ~~pass~~ successfully pass this test[[,]] and consequently they permit ~~to calibrate~~calibration of this biological test by comparison.

Test S3

The thus selected strains through the hereabove defined baker's yeast selections are then subjected to a third series of selection tests, S3, which consist in the determination of their resistance against the stress caused by freezing, in other words their qualification for the preparation of baker's yeasts which can be used in the

manufacture of frozen dough pieces intended for sweet rolls and for sweet Danish pastries.

The compositions of the sweet rolls and the sweet Danish pastries used in ~~that~~ the third series of tests are as follows:

1) Rolls containing 6 % of HFCS (high fructose corn syrup) dry matter or 10 % of sucrose:

- flour	100.0
- water	55.0 %
- yeast expressed in dry matter	1.86 %
- HFCS expressed in dry matter	6.0 %
	(or sucrose 10.0 %)
- fats	5.0 %
- salt	2.0 %
- dough improver	2.0 %

2) Sweet Danish pastries:

- flour	100.0
- water	46.0 %
- yeast expressed in dry matter	2.72 %
- sucrose	18.0 %
- fats	13.0 %
- pulverulent lactoserum	4.0 %
- salt	2.0 %
- dough improver	2.0 %

The percentages are expressed in what is called "baker's percent", that is to say in weight with respect to 100 parts of the total flour used.

The flour used is a US flour having a high gluten content well adapted to bread-making comprising a deep-frozen step.

The dough improver used for the rolls and for the Danish pastries brings gluten, diacetyltartaric esters of monoglycerides (DATEM), ascorbic acid, alpha-amylases and

hemicellulases, in amounts permitting to obtain optimized dough pieces for deep frozen and long storages at –20°C.

The conditions of the manufacture of the frozen doughs intended for rolls and for Danish pastries, those of the storage of the frozen doughs until thawing of the frozen doughs and those of the tests to which ~~are subjected~~ the dough pieces are subjected are as follows:

- mixing,
- temperature of the dough at the end of mixing: 19°C,
- separation in balls of 100 g, in a room whose temperature is 19°C,
- beginning of deep-freezing 35 minutes after the end of mixing,
- deep-freezing during 35 minutes at –30°C which provides a temperature at the center of dough pieces of –5°C,
- storage at –20°C during 100 days,
- thawing within 20 hours at 0°C at the end of each of the storages of 100 days,
- determination after thawing of the total gas release using the zymotachygraphe Chopin® during 2 hours and 30 minutes at 27°C,
- proofing and measuring of the proof time at 35°C on three dough pieces or balls,
- baking and appreciation of volume and of the scoring of obtained rolls or pastries and notably verification of the absence of any bad taste or any off-flavors.

Within the framework of these tests, baker's yeasts were selected which provided dough pieces of sweet Danish pastries obtained as here-above disclosed and thawed after 100 days at - 20°C, which showed the following performances:

- total gas release measured using a zymotachygraphe CHOPIN® in 2 hours and 30 minutes at 27°C at least higher by 20 % with respect to that of the pieces obtained under the same conditions using the control yeast conventionally manufactured starting from the strain CNCM I-2412 and stored in the same manner,
- proof time lower by at least 10 % with respect to that of dough pieces obtained and stored under the same conditions but using the control yeast,
- absence of any bad taste or any off-flavors.

The tests carried out with the dough pieces corresponding to the formulations of sweet rolls ~~permit to~~ confirm the selection because they ~~permit to~~ verify that the ~~searched differences~~desired properties are also obtained with a wide range of sweet pastries.

The two strains selected at the end of these three systematical selection tests are the two strains here-above identified, i.e., the strains deposited at the CNCM under the numbers I-2421 and I-2422.

These two strains have been selected because the yeasts manufactured starting from these strains ~~gave~~ give rise with respect to the control yeast to the greatest differences in all the tests of frozen and thawed doughs.

The yeasts obtained with these two selected strains ~~permitted to obtain~~allowed for the production of frozen sweet Danish pastry pieces which, when thawed after 100 days at - 20°C, provided a total gas release which was higher by at least 30 % when measured with the zymotachygraphe CHOPIN® and a proof time lower by at least 20 %, in fact by 25 %, with respect to the control yeast used under the same conditions.

These two strains ~~permit to obtain~~are used to produce yeast creams and fresh yeasts which show a good aptitude ~~to keep~~for maintaining their properties during storage. These two selected strains CNCM I-2421 and I-2422 also present ~~the~~ interesting properties ~~consisting in~~consistent with the fact that they ~~are responding~~ respond well to cultivation processes with discontinuous inflow of molasses as those disclosed in ~~the~~ UK ~~patent~~ Patent No. 1,539,211 or US ~~patent~~ Patent No. 4,328,250 with the view of improving the performances of the yeasts ~~on~~ in sweet doughs and especially in the fact that they are keeping these higher performances on sweet dough, due to the use of the said cultivation processes, in sweet dough pieces thawed after several months of storage at - 20°C. Preferably, the baker's yeasts according to the invention are obtained using such kind of cultivation processes of adaptation to the fermentation of sweetened doughs.

These two strains CNCM I-2421 and I-2422 also have the property ~~to give~~of giving frozen intermediate dry yeasts the use of which is particularly interesting for the making of sweet frozen doughs.

The frozen intermediate active dry yeasts are defined as frozen dry yeasts in the form of particles, having an intermediate dry matter, i.e., a dry matter content of from 70 to 80% in weight, preferably 72 to 78%, still preferably 74 to 78%. The ~~said~~ frozen intermediate active dry yeasts can be made as described in European ~~patent~~ Patent No. 0237427 B2 corresponding to Canadian ~~patent~~ Patent No. 1,299,435 or corresponding to Australian ~~patent~~ Patent No. 609 030 (document No. AU-B-69781/87), the entire disclosures of which are incorporated by reference.

Different trials carried out with strain CNCM I-2421 cultivated according to a process with discontinuous inflow of molasses during the whole or part of the last cycle of multiplication, have led to frozen intermediate dry yeasts between 70 and 80 % dry matter, preferably between 72 and 78 % dry matter, giving the following gas releases in tests A_1, A_5, A_6 described in US ~~patent~~ Patent No. 5,741,695:

test A_1 170 ml to 190 ml in two hours

test A_5 110 ml to 130 ml in two hours

test A_6 115 ml to 140 ml in two hours.

In these tests A carried out with frozen intermediate active dry yeasts, the 160 mg of yeast solid content (tests A_1 or A_5) or the 320 mg of yeast solid content (test A_6) of frozen intermediate dry yeast are thawed during one hour at room temperature before being mixed with the 15 ml of water as described column 5 of US ~~patent~~ Patent No. 5,741,695.

These two strains CNCM I-2421 and CNCM I-2422 can be characterized by using the identification technique of yeast strains using the Polymerase Chain Reaction and based on the amplification of the inter delta zones of the retrotransposon TY1, ~~and~~ which is disclosed in the Article "Identifications of Yeast Strains Using the Polymerase Chain Reaction" by F. Ness, F. Lavallée, D. Dubourdieu, M. Aigle and L. Dulau, published in J. Sci. Food Agric. 1993, 62, 89-94.

In that respect it appears from ~~the single figure or figure~~Figure 1 which shows ~~in 1, 2 and 3~~ the electrophoresis profiles of amplified DNA sequences of strains L17 and L35 and a profile of digested DNA used as a molecular weight marker, that the strain L17 (CNCM I-2421) presents two supplemental bands with respect to the strain L35

(CNCM I-2422). Taking into consideration the above-indications, it appears that the present invention relates as a new industrial product not only to the baker's yeasts hereabove defined but also to the strains deposited under the numbers CNCM I-2421 and I-2422.

The present invention also relates on the one hand to the use of the two strains CNCM I-2421 and CNCM I-2422 and on the other hand to the use of similar strains to these two strains, for the preparation of baker's yeasts according to the invention in the form of yeast creams, fresh compressed yeasts and active dried yeasts, preferably in the form of frozen intermediate active dry yeasts. It relates also to the new baker's yeasts able to be thus obtained. Similar strains to the two strains CNCM I-2421 and CNCM I-2422 are defined as the strains sharing all the common properties to these two strains and/or as the strains able to be selected by the three series of systematical selection tests hereabove disclosed, i.e., the baker's yeast strains which pass successfully the ~~said~~ three systematical selection tests described herein.

As already indicated, baker's yeast strains which have the property ~~to be~~of being resistant to the stress caused by freezing were known, but these baker's yeast strains cannot be used without formation of off-flavors in the manufacture of bakery products containing cinnamon.

This default can be corrected and is corrected by the deletion or disruption or inactivation in the genome of ~~said~~ the baker's yeast strains of the PAD1 gene(s) encoding phenylacrylic acid decarboxylase, the enzyme which permits the decomposition of cinnamic acid.

The PAD1 gene is described and characterized entirely by Clausen et al. (1994), Gene 142: 107-112 in an article entitled "PAD1 encodes phenylacrylic acid decarboxylase which confers resistance to cinnamic acid in Saccharomyces cerevisiae." This PAD1 gene is also called Phenolic Off Flavors gene or POF gene.

Such a targeted inactivation of the PAD1 gene can be obtained by conventional gene replacement methods (see Rothstein in Guthrie C. and Fiur GR (editors) Guide to Yeast Genetics and Molecular Biology in Methods in Enzymology vol. 194: 281-301) or by employing an integration/excision DNA cassette as described in ~~the~~ European ~~patent~~

~~application~~Patent Application No. 0 994 192 or in the corresponding US ~~patent application~~Patent Application Serial No. 09/415,216 filed on October 12th, 1999, ~~and having as title~~entitled "Yeast ~~transformation cassette~~Transformation Cassette" or still the Australian ~~patent application~~Patent Application No. 53572/99, the entire disclosures of which are incorporated by reference.

These patent applications which are incorporated herein by reference disclose integration/excision DNA cassettes which permit the total or partial deletion of ~~a~~ the same gene in Saccharomyces cerevisiae, leaving in the host strain only yeast DNA. In order to disrupt the PAD1 gene(s), ~~it is constructed~~ integration/disruption cassette(s) CAS-PAD are constructed on the same principle of the integration/disruption cassettes CAS-SUC disclosed in the example 4 of these patent applications and the ~~said~~ cassette(s) CAS-PAD are used to delete or disrupt the alleles of the PAD1 gene by integration(s)/excision(s) as disclosed in the ~~said~~ example 4. This strategy consisting ~~in~~ of the use of the said integration/excision DNA cassette(s) is preferred ~~by the applicants as~~because it allows ~~to inactivate~~for the inactivation of all copies of the PAD1 gene in a strain containing more than one copy as it is often the case for industrial strains. In addition, this strategy leads to the construction of strains, in which the selectable marker has been eliminated.

This elimination of the selectable marker is very important. Today no baker's yeast is obtained by multiplication of a genetically modified strain and it is generally admitted that only a genetically modified strain stable and without a selectable marker could be acceptable as a baker's yeast strain.

The present invention relates to ~~the~~ new baker's yeast strains obtained (or modified) by clean deletion of the PAD1 gene(s) encoding phenylacrylic acid decarboxylase. A clean gene deletion or inactivation is defined as a genetical modification which cuts out the expression of the deleted or inactivated gene(s) (i.e., in the frame of present invention the PAD1 gene(s)), without leading to the expression of a heterologous gene, and preferably without leading to the production of any new compound toward a natural yeast mutant strain which has its said gene(s) disrupted or inactivated.

Another preferred strategy in order to obtain baker's yeast strains which have their ~~gene(s)~~ PAD1 gene(s) inactivated, consists in mutating baker's yeast strains intended to be modified, by a classic mutagenic treatment giving a cell survival ratio of about 20 to 30% and selecting in a first time the mutated strains which do not show ~~no~~ any phenylacrylic acid decarboxylase activity in the presence of cinnamic acid and/or ferulic acid and/or coumaric acid, and notably which show no production of off-flavors (volatile phenols and/or styrene). In a second time, it is verified on the selected mutated cells presenting this property, the no-expression of ~~gene(s)~~ PAD1 gene(s) by conventional technics as Northern hybridization (described in Molecular Cloning, a laboratory manual, second edition, Sambrook, Fritsch, Maniatis); and it is verified that the ~~said~~ mutated (mutant) strains not expressing their gene(s) PAD1 have kept the whole of their interesting properties as baker's yeast strains.

Preferably the present invention relates to baker's yeast strains resistant to the stress caused by freezing and modified by clean deletion (disruption) of the PAD1 gene(s). The starting or host strains are known baker's yeast strains resistant to the stress caused by freezing, such as the baker's yeast strains ~~giving~~ used to produce the European baker's yeasts ~~performing on~~for use with frozen doughs cited hereabove, ~~the said baker's yeast strains being~~which are modified by clean deletion of the PAD1 gene(s). Still preferably, the ~~said~~ starting or host known baker's yeast strains selected in order to be modified by clean deletion of the PAD1 gene(s) contained in their genome, pass successfully the first selection test and the third series of selection tests hereabove disclosed, and the modified strains pass successfully the three series of selection tests hereabove disclosed.

~~Indeed, the~~The present invention relates also to the same or equivalent baker's yeast strains obtained by mutation the ~~gene(s)~~ PAD1 gene(s) of which is(are) inactivated. ~~Indeed, the~~The ~~said~~ mutation process leads to a clean inactivation as hereabove defined. ~~Indeed,~~ In addition, the present invention relates to all the new baker's yeast strains obtained by clean inactivation of their PAD1 gene(s) whatever method is ~~the~~ used ~~mean of~~ for inactivation.

The present invention relates also to new baker's yeasts having the ~~new~~ properties hereabove disclosed, which are obtained by a process comprising the use of any of the ~~said~~ new modified or mutated baker's yeast strains hereabove disclosed. In a general manner, the present invention relates to the use of the hereabove disclosed modified or mutated baker's yeast strains, obtained by inactivation of the PAD1 gene(s), for the preparation of baker's yeasts in the form of yeast creams, fresh compressed yeasts, frozen intermediate active dry yeasts, and active dry yeasts.

Still, in a general manner, the present invention relates to a process for the preparation of new baker's yeasts according to the invention comprising the conventional cultivation or multiplication of a selected starting strain. Preferably, the new baker's yeasts according to the invention are obtained by the use of a special cultivation process corresponding to a fed-batch process comprising a discontinuous inflow of molasses during the whole or part of the last cycle of cultivation. The invention ~~has~~ also has as an object, new baker's yeasts having the new properties hereabove disclosed and obtained or able to be obtained by a process comprising a discontinuous inflow of molasses during the whole or part of the last cycle of cultivation.

Preferably the ~~said~~ selected starting strain is strain CNCM I-2421 or I-2422, but the ~~said~~ selected strain can be also a similar strain to strains CNCM I-2421 and CNCM I-2422, i.e., a strain sharing all the common properties to these two strains and/or able to be selected as hereabove disclosed, or also a baker's yeast strain obtained by clean inactivation of the PAD1 gene(s).

The present invention also relates to new frozen intermediate active dry yeast products, having between 70 and 80% dry matter, preferably between 72 and 78% dry matter, still preferably between 74 and 78% dry matter. The ~~said~~ frozen intermediate active dry yeasts are preferably in the form of rod shaped flowing particles of a diameter less than 3mm, preferably less than 1mm, and they are preferably obtained by a gentle drying of a fresh baker's yeast (i.e., a baker's yeast between about 30% and about 35% dry matter) until the desired dry matter and a freezing by fluidization. The ~~said~~ frozen intermediate active dry yeasts preferably have ~~preferably~~ the following properties:

- very good ~~performances~~ performance in tests A_1 A_5 and A_6 carried out with the fermentometer of Burrows and Harrison, i.e.,
  - in test A_1 gas release between 170 ml and 190 ml in two hours
  - in test A_5 gas release between 110 ml and 130 ml in two hours
  - in test A_6 gas release between 115 ml and 140 ml in two hours.
- they are resistant to the stress caused by freezing when they are used in sweet frozen doughs, i.e., when they are used for the making of sweet Danish pastry doughs comprising 18% sucrose with respect to the flour used and comprising fats, the following characteristics are obtained:
  - on the one hand, the total gas release recorded with the zymotachygraphe Chopin® on a dough piece frozen and thawed after at least 100 days is higher by at least 20% than the total gas release recorded under the same conditions when using the same amount of yeast dry matter of a conventional control fresh yeast obtained starting from strain CNCM I-2412
  - on the other hand, the proof time of the said sweet Danish pastry doughs, frozen and thawed after at least 100 days is lower by at least 10% than that measured under the same conditions when using the same amount of yeast dry matter issued of the said conventional control fresh yeast.
- they do not give rise to the appearance of bad taste or of off-flavors in the presence of cinnamon.

The ~~said~~ new frozen intermediate active dry yeasts in fine free flowing particles can contain free flowing agents or anticaking agents as silica and silicates. It can also contain ~~also~~ drying processing aids and/or rehydration agents such as sorbitan monostearate, gums, carboxy-methyl-cellulose.

The ~~said~~ new frozen intermediate active dry yeasts according to the invention have the advantage ~~to keep at least 6 months, preferably one year,~~ of maintaining their initial properties for at least 6 months, and more preferably one year.

In other words the present invention relates to new baker's yeasts ~~according to the invention~~ in the form of particles of frozen active dry yeast having an intermediate dry matter, i.e., a dry matter between 70% and 80%, preferably between 72% and 78%.

Preferably the ~~said~~ new baker's yeasts according to the invention in the form of frozen active dry yeast are obtained or obtainable by a process comprising the use as starting strain of one of the strains belonging to the group of the strains CNCM I-2421 and I-2422 and of the similar strains to these two strains CNCM I-2421 and I-2422 and of the baker's yeast strains obtained by inactivation of the PAD1 gene(s). Still preferably the ~~said~~ process comprises a discontinuous inflow of molasses during the whole or part of the last cycle of multiplication. Still more preferably the new baker's yeasts according to the invention, in the form of frozen intermediate dry yeasts, have the hereabove defined gas releases in hereabove defined tests A_1, A_5 and A_6.

The present invention also relates to a process for the preparation of baker's yeasts comprising the use as starting strain of one of the strains of the group comprising the strains deposited according to the Budapest Convention with the "Collection Nationale de Cultures de Microorganismes", Institut Pasteur, 28 rue du Docteur Roux, 75724 PARIS CEDEX 15, under the numbers I-2421 and I-2422.

The present invention also relates to a process for the preparation of baker's yeasts comprising the use as starting strain of one of the strains selected from the group of the strains similar to the two strains I-2421 and I-2422 and the baker's yeast strains obtained by clean inactivation of the PAD1 gene(s).

Preferably in the ~~said~~ process, the strain chosen among the group comprising the strains I-2421 and I-2422 and the strains similar to the two strains I-2421 and I-2422 and the hereabove defined strains obtained by clean inactivation of the PAD1 gene(s) is cultivated according to a fed-batch process comprising a discontinuous inflow of molasses during the whole or part of the last cycle of cultivation, i.e., during the last hours before the yielding of the yeast cells in order to obtain commercial cream yeast, fresh compressed or crumbled yeast, active dry yeast, intermediate frozen active dry yeast.

In fact, on the one hand the process according to the present invention for the preparation of new baker's yeasts according to the invention is ~~firstly original~~novel, due to the use of a selected starting strain as hereabove disclosed, and on the other hand it can use the techniques conventionally used in the manufacture of baker's yeasts.

However, preferably ~~the said process uses~~ a cultivation process comprising a discontinuous inflow of molasses is used.

Concerning any details relating to these techniques, reference is made to the manual "YEAST TECHNOLOGY", Reed and Peppler, The AVI PUBLISHING, 1973, or to the second edition of that manual by Reed and Nagodawithana, An AVI book published by VAN NOSTRAND REINHOLD, 1991, which are incorporated by reference or still preferably to ~~the~~ US ~~patents~~ Patent Nos. 4,328,250, 4,396,632 and 5,741,695 and to the UK ~~patent~~ Patent 1,539,211, and to the European ~~patent~~ Patent No. 0 237 427 B2 or the Australian ~~patent~~ Patent No. 609030, the entire disclosures of which are also incorporated by reference.

~~Indeed, the~~The present invention relates also to new baker's yeasts obtained or able to be obtained (= obtainable) according to the hereabove disclosed processes.

The present invention relates finally to the use of baker's yeasts according to the invention for the manufacture of bread-making doughs aromatized (flavored) with cinnamon and/or for the manufacture of frozen dough pieces especially on the basis of sweetened doughs. Notably, it relates on the one hand to a process for the manufacture of breadmaking doughs aromatized with cinnamon, and on the other hand to a process for the manufacture of frozen dough pieces, preferably sweet frozen dough pieces, comprising the use of new baker's yeasts according to the present invention. Preferably the invention relates to the said dough making processes comprising the use of a baker's yeast belonging to the group of the new baker's yeasts having good general performance in not delayed bread-makings, resistant with respect to the stress caused by freezing when they are used in sweetened doughs, and not giving rise to the appearance of off-flavors in the presence of cinnamon and of the new baker's yeasts obtained or obtainable by the process comprising the use as starting strain of one of the strains of the group comprising the strains CNCM I-2421 and I-2422, and the similar strains to these two strains, i.e., the strains sharing all the common properties to the said strains I-2421 and I-2422 and/or which are able to be selected as disclosed hereabove, and the baker's yeast strains obtained by clean inactivation of the PAD1 gene(s), being understood in a general manner that preferably these modified or

mutated baker's yeast strains are also resistant to the stress caused by freezing, and still preferably pass successfully the three series of selection tests hereabove disclosed. Preferably, these new baker's yeasts are in the form of frozen intermediate active dry yeasts.